"The Action of Choline, Neurine, Muscarine, and Betaïne on Isolated Nerve and upon the Excised Heart." By A. D. Waller, M.D., F.R.S., and S. C. M. Sowton. Received June 12,—Read June 18, 1903.

In connection with the identification by Halliburton and Mott of choline in morbid cerebro-spinal fluid, we compared the action upon isolated nerve and upon the excised heart of the four closely related organic bases: choline, $C_5H_{15}NO_2$; neurine, $C_5H_{13}NO$; muscarine, $C_5H_{13}NO_2$; and betaïne, $C_5H_{13}NO_3$; we have also, thanks to the kindness of Professor Wright and of Mr. Plimmer, taken occasion to examine in a similar manner certain pathogenic toxines, viz.: snake venom (Calmette), diphtheria toxin, and tetano-toxin.

According to previous investigators, muscarine is powerfully toxic, arresting the heart in diastole (Schmiedeberg); neurine has an action resembling that of muscarine; choline (which formerly was not distinguished from neurine) has a less powerful action than that of neurine, and betaïne is considered to be an inert substance. (With regard to their possible action upon nerve, there are, so far as we know, no definite observations.) The direct action of muscarine upon nerve was incidentally examined by one of us in a general survey of the action upon nerve of a series of vegetable alkaloids; that of choline and neurine was examined in comparison with a cerebro-spinal residue and briefly reported upon at the Cambridge Congress of Physiology (1897).

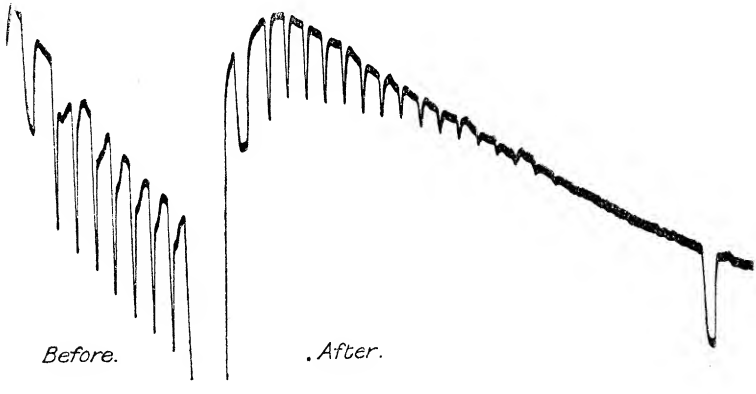
At that time, confining ourselves to a procedure in which the nerve was submitted to observation for a period not exceeding one hour, interrupted by a period of immersion of one minute duration, we found that muscarine was to be ranked with alkaloids possessing "little or no action" upon nerve, with, however, the express reservation that "such a statement must not be taken as committing us to a denial of any action whatever by the drug acting in stronger solution or for a longer period."

And in point of fact, muscarine which, under the conditions systematically observed by us at the outset of these observations, is to be classified as inactive, is manifestly active (*a*) in stronger solution for the same short period of immersion, and (*b*) in the same weak solution for a longer period of immersion. The former of these two statements is illustrated by fig. 1 (3319), giving the effect of muscarine nitrate in 10-per cent. solution acting for one minute; the latter statement by *e.g.*, fig. 4 (*vide infra*), which represents the course of an observation extending over 60 hours, in which two nerves were three times submitted to prolonged immersion in a 1-per cent. solution of muscarine hydrochloride.

As regards choline, neurine, and the cerebro-spinal residue our report of a summary examination of these three bodies was to the effect that choline as compared with neurine was inert, that cerebro-spinal residue was inert while fresh, but became active when oxidised, and that "as regards an action upon isolated nerve the order of efficacy of the samples in our possession was (1) neurine, (2) muscarine, (3) choline."*

This result, although accurate for the particular samples in our hands, was, however, vitiated by an error in their description. The so-called "neurine hydrochloride" of our first experiments

FIG. 1 (Muscarine nitrate).



3313 to 3317, was in reality the base neurine, which in 25-per cent. solution has a basic reaction requiring for its neutralisation 2 vols. of normal acid. The effects of 4, 2, 1, and $\frac{1}{2}$ and $\frac{1}{4}$ per cent. solutions of neurine are, therefore, partly or wholly basic effects by 0·32, 0·16, 0·08, 0·04, and 0·02 solutions of normal alkali. These effects were in fact such as we are accustomed to expect from other basic solutions such as potash or soda, of strength ranging from 0·20 to 0·05 normal (acid and alkali).

A strict comparison between the two bodies requires the use of both bodies as bases, neither of which conditions we have yet found means to fulfil. For, on the one hand, neurine when neutralised by an acid (*e.g.*, hydrochloric), is decomposed to an ammonium salt, etc., on the other, choline as base is extremely unstable.

A fresh series of experiments for determining the relative effects of choline and neurine upon isolated nerve was made in the summer of 1900. We obtained from Messrs. Merck the four

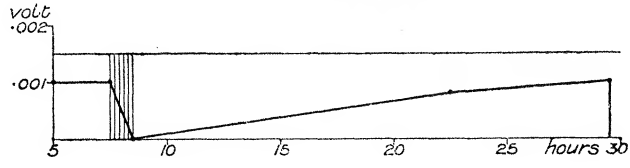
* Sowton and Waller, Internat. Physiol. Congress, Cambridge, 1898, 'Journ. of Physiol.,' vol. 23, suppl., p. 35.

substances: choline and neurine pure, choline hydrochloride, and neurine hydrochloride. These were used in solutions of 10 per cent. or less, and in the case of the pure substances both unneutralised and neutralised solutions were tried. Series of nerves were immersed (*a*) for 1 minute, (*b*) for 30 minutes, the experiments being photographically recorded. At the end of each experiment the nerve was replaced in physiological saline, and some hours later, usually the next morning, having received a new transverse section, it was again tested in order to ascertain whether any effect that had been recorded was permanent or merely temporary.

In making up solutions of choline the question arose as to whether physiological saline or distilled water should be used. Saline being inadmissible in the case of neurine, the comparisons to be made would be more fair, it seemed, if all solutions were made with distilled water.

In working, however, with choline hydrochloride, a solution was made up with saline, and a second one with distilled water, in order by comparing the effects of the two, to estimate roughly what proportion of any effect obtained should be attributed to the action of the distilled water. In the course of former experiments some attention had already been paid to the separate action of distilled water upon nerve, and immersion for 30 minutes was found to diminish its electromobility. But between the two choline hydrochloride

FIG. 2.—Distilled water.



solutions no great difference of effect could be noticed. After this first trial the solutions of both choline and neurine were made up with distilled water only.

A 1-minute bath of choline hydrochloride at 10 per cent. has little or no effect upon nerve response. A 30-minute bath of the same solution diminishes the deflection, but soakage for some hours in physiological saline restores completely the electromobility of the nerve. The drug then may be said to be active in strong solutions applied for a considerable time—it cannot be characterised as "toxic."

With neurine hydrochloride in 10 per cent. solution the effect of a 1-minute bath is diminution of the electromobility of the nerve, similar in degree to that observed after 30 minutes of choline hydrochloride of the same strength, but the nerve treated with neurine gives after

Table A.—Choline Hydrochloride

No. of plate.	Substance.	Solution.	Time.	Remarks.
			mins.	
3601	Choline hydrochloride	10 p. c. in distilled water	1	No diminution of response.
3602	,, ,, ...	,, saline	1	Little or no effect.
3603	,, ,, 2nd bath	,, distilled water	30	Diminution by 0·00023 volt in 20 mins. (0·00088 to 0·00065 volt) (nerve of 3601).
3604	,, ,, ...	,, ,,	1	Little or no effect.
3605	,, ,, ...	,, ,,	1	Little or no effect.
3606	,, ,, 2nd bath	,, ,,	30	Marked diminution, by 0·00054 volt within 20 mins. (0·00075 to 0·00021 volt) (nerve of 3604).
3607	,, ,, ...	,, saline	30	Diminution to trace within 20 mins.
3608	,, ,, ...	,, ,, ...	30	Diminution by 0·00021 volt within 20 mins. (0·00054 to 0·00033 volt).
3609	,, ,, 2nd bath	,, distilled water	30	Diminution by 0·00036 volt within 20 mins. (0·00058 to 0·00022 volt).

The effect, where effect was observed, was in these cases gradual. Two experiments made when the solution was five or six weeks old, show more rapid action, but in these cases also the effect was only temporary, both nerves giving good deflections the next day.

3679	Choline hydrochloride (old)	10 p. c. in distilled water	30	Abolition of response.
3680	,, ,, ...	,, ,,	30	Trace left, abolition complete within 6 mins.

Table B.—Neurine Hydrochloride.

No. of plate.	Substance.	Solution.	Time.	Remarks.
			mins.	
3645	Neurine hydrochlorine	10 p. c.	1	Diminution by 0·00108 volt within 25 mins. (0·0018 to 0·00072 volt).
3646	,, ,, ...	,,	1	Little or no effect.
3647	,, ,, 2nd bath	,,	30	Abolition complete and final (nerve of 3645).
3648	,, ,, ,, ...	,,	30	Abolition complete and final (nerve of 3646).
3649	,, ,, ...	,,	1	Marked diminution, by 0·00046 volt within 25 mins. (0·00066 to 0·0002 volt).
3650	,, ,, ...	,,	30	Trace for first few minutes after bath, then final abolition.
3651	,, ,, ...	,,	1	Diminution, by about 0·0005 volt within 25 mins. (0·001 to about 0·0005 volt). Deflection just visible next day.
3652	,, ,, ...	,,	1	Diminution marked, 0·0009 volt within 20 mins. (0·001 to 0·001 volt). No response next day.
3653*	,, ,, ...	,,	1	Augmentation by 0·00033 volt (0·00043 to 0·00074 volt). Good deflection next day.
3654	,, ,, ...	,,	1	Diminution by 0·00041 volt within 25 mins. (0·00078 to 0·00037 volt). Very small deflection next day.
3655	,, ,, ...	,,	1	Diminution by 0·00005 volt within 25 mins. (0·008 to 0·0003 volt). No response next day.
3656	,, ,, ...	,,	30	Abolition, complete and final.
3657	,, ,, ...	,,	30	Diminution to a trace within 25 mins. No recovery.
3658	,, ,, ...	,,	30	Abolition, complete and final.

long soaking in physiological saline either a much-reduced deflection or none at all. One exceptional case was recorded: on Plate 3653, the effect of neurine hydrochloride for 1 minute is marked augmentation, and the nerve on being tested the next day gave a good deflection.

With a 30-minute bath of neurine hydrochloride the effect is usually abolition immediate and final. In two cases a very small deflection was recorded after the bath (Plates 3650, 3657), but in no case was there any subsequent recovery.

Pure choline, giving an alkaline reaction, was first tested in its non-neutralised state. Immersion of the nerves for 1 minute had always some effect, though not a marked one. Out of six records taken, three show slight diminution, the other three slight augmentation. Four of the nerves were tested the next day and gave deflections, three of them large ones. The two other nerves were subjected the same day to the longer bath—30 minutes—with the result recorded on plates 3666, 3667, viz., diminution to a mere trace within 20 minutes and abolition within 12 minutes; both, however, gave small deflections on being tested the following day. In three other experiments with a bath of 30 minutes the deflection was reduced, markedly in two cases—but there was partial recovery by the next day. In the remaining case the diminution was less marked on the record, but there was no after recovery.

To obtain a 10-per cent. neutralised solution of choline, 1 vol. 20 per cent. choline was mixed with 1 vol. sulphuric acid n/10. The effect upon nerve was less marked than in the case of pure choline solution, which had shown one example of final abolition; the neutralised solution gave, for the most part, diminution, but the recovery was apparently more complete than with pure choline. In one experiment (3672) the deflection next day was very small.

Pure neurine non-neutralised was found as in former experiments to be very toxic to nerve. At 10, 8, 5, and 4 per cent., immersion for 1 minute abolished all response. (The one exceptional case recorded, Plate 3612, where the effect was only slight diminution, we cannot attempt to explain; at 2 per cent.—1 minute bath—the deflection was abolished, but there was partial recovery by the next day.) At 1 per cent.—1 minute—there was diminution or abolition with recovery.

The results obtained with neutralised neurine were not very satisfactory. The neurine was received from Messrs. Merck in a 25-per cent. solution and required for the neutralisation 2 vols of normal sulphuric acid. A glance at the table of experiments will show how uncertain are the effects as compared with those of neurine hydrochloride. For instance, Nos. 3619 and 3620 were a pair of nerves subjected to a 1-minute bath of 8 per cent. neutralised neurine; there was little or no effect upon either nerve, they were then further

Table C.—Choline (Alkaline).

No. of plate.	Substance.	Solution.	Time.	Remarks.
			mins.	
3659	Choline, not neutralised	10 p. c.	1	Diminution by 0·00038 volt within 28 mins. (0·00097 to 0·00059 volt). Small deflection next day.
3660	,, ,, ...	,,	1	Diminution by 0·00024 volt within 20 mins. (0·00077 to 0·00053 volt). Good deflection next day.
3661	,, ,, ...	,,	30	Marked diminution by 0·00098 volt within 20 mins. (0·0011 to 0·00012 volt). Deflection next day.
3662	,, ,, ...	,,	1	Augmentation by 0·0003 volt (0·0007 to 0·001 volt). Nerve used again.
3663	,, ,, ...	,,	1	Augmentation by 0·00026 volt (0·00069 to 0·00095 volt). Nerve used again.
3664	,, ,, ...	,,	1	Diminution by 0·00012 volt (0·00075 to 0·00063 volt). Good deflection next day.
3665	,, ,, ...	,,	1	Augmentation by 0·00014 volt (0·00089 to 0·00103 volt). Good deflection next day.
3666	,, ,, 2nd bath ..	,,	30	Diminution to trace within 20 mins. Small deflection next day (nerve of 3662).
3667	,, ,, ,, ..	,,	30	Abolition within 12 mins. Deflection next day (nerve of 3663).
3668	,, ,, ...	,,	30	Diminution by 0·00038 volt within 20 mins. (0·00095 to 0·00057 volt). Small deflection next day.
3669	,, ,, ...	,,	30	Marked diminution by 0·00052 volt (0·0007 to 0·00018 volt). No deflection next day.

Table C—*continued.*

No. of plate.	Substance.	Solution.	Time.	Remarks.
			mins.	
3670	Choline, neutralised	10 p. c.	1	Slight augmentation by 0·0001 volt (0·0006 to 0·0007 volt). Good deflection next day.
3671	,, ,, ...	,,	1	Diminution by 0·00014 volt (0·00075 to 0·00061 volt). Good deflection next day.
3672	,, ,, ...	,,	30	Primary augmentation, no diminution on plate, but very small deflection next day.
3673	,, ,, ...	,,	30	Diminution to a trace within 20 mins. Good deflection next day.
3674	,, ,, ...	,,	30	Primary augmentation. Good deflection next day.
3675	,, ,, ...	,,	30	Primary augmentation, then diminution by 0·0008 volt within 20 mins. Not tested next day.
3676	,, ,, ...	,,	30	Diminution by 0·0007 volt within 20 mins. (0·0025 to 0·0018 volt). Not tested next day.
3677	,, ,, ...	,,	30	Diminution. Good deflection next day.
3678	,, ,, ...	,,	30	Diminution. Good deflection next day.
3681	,, ,, 2 weeks old	,,	1	Augmentation by 0·0004 volt (0·001 to 0·0014 volt).
3682	,, ,, ...	,,	1	Slight augmentation by 0·0001 volt (0·0017 to 0·0018 volt). Good deflection next day (nerve used on 3683).
3683	,, ,, 2nd bath	,,	30	Diminution by 0·00084 volt (0·00134 to 0·0005 volt) within 20 mins. Very small deflection 8 hrs. later and next day.
3684	,, ,, ...	,,	30	Abolition within 20 mins. Deflection next day.
3685	,, ,, ...	,,	30	Diminution by 0·00051 volt within 20 mins. (0·00089 to 0·00038 volt). Deflection next day.

Table D.—Neurine (Alkaline).

No. of plate.	Substance.	Solution.	Time.	Remarks.
			mins.	
3610	Neurine, not neutralised	10 p. c.	1	Abolition immediate and final.
3611	,, ,, ...	,,	1	Ditto.
3629	,, ,, ...	8 p. c.	1	Ditto.
3630	,, ,, ...	,,	1	Ditto.
3612*	,, ,, ...	5 p. c.	1	Slight diminution. Not tested again.
3613	,, ,, ...	,,	1	Abolition immediate and final.
3638	,, ,, ...	,,	1	Ditto.
3641	,, ,, ...	4 p. c.	1	Ditto.
3642	,, ,, ...	,,	1	Ditto.
3643	,, ,, ...	2 p. c.	1	Abolition within 15 mins. Small deflection next day.
3644	,, ,, ...	,,	1	Abolition within 15 mins. Small deflection next day.
3639	,, ,, ...	1 p. c.	1	Abolition within 10 mins. Good deflection next day.
3640	,, ,, ...	,,	1	Diminution. Deflection next day.

Table D—*continued.*

No. of plate.	Substance.	Solution.	Time.	Remarks.
			mins.	
3614	Neurine, neutralised	5 p. c.	1	Little or no effect. The nerve was afterwards submitted to non-neut. 5 p. c. solution, which killed it.
3315	,, ,, ...	8 p. c. (about)	1	Diminution by 0·001 volt within 25 mins. (0·002 to 0·001 volt).
3616	,, ,, ...	,,	1	Diminution by 0·00011 volt (0·00033 to 0·00022 volt).
3617	,, ,, ...	,,	30	Immediate abolition. But gives deflection next day.
3618	,, ,, ...	,,	30	Immediate abolition.
3619	,, ,, ...	,,	1	Little or no effect. Nerve used again, pl. 3621.
3620	,, ,, ...	,,	1	Little or no effect. Nerve used again, pl. 3622.
3621	,, ,, 2nd bath	,,	30	Diminution by 0·0005 volt within 20 mins. (0·001 to 0·0005 volt). Good deflection next day.
3622	,, ,, ,, ...	,,	30	Abolition within 5 mins. No recovery.
3623	,, ,, ...	,,	30	Abolition immediate and final.
3624	,, ,, ...	,,	30	Ditto.
3625	,, ,, ...	4 p. c. (about)	30	Very slight increase on record. No deflection next day.
3626	,, ,, ...	,,	30	Diminution by 0·0005 volt within 25 mins. (0·0015 to 0·001 volt).
3627	,, ,, ...	,,	30	Slight augmentation. Deflection next day.

Table D—*continued*

No. of plate.	Subject.	Solution.	Time.	Remarks.
			mins.	
3628	Neurine, neutralised	4 p. c. (about)	30	Slight augmentation. Deflection next day.
3631	,, ,, ...	2 p. c.	30	Slight augmentation. No deflection next day.
3632	,, ,, ...	,,	30	No effect. Small deflection next day.
3633	,, ,, ...	,,	30	Diminution by 0·0006 volt within 20 mins. (0·00082 to 0·00022 volt). No deflection next day.
3634	,, ,, ...	,,	30	Diminution by 0·0005 volt within 20 mins. (0·0013 to 0·0008 volt). Deflection first visible next day.
3635	,, ,, ...	,,	30	Slight augmentation followed by slight diminution. Good deflection next day.
3636	,, ,, ...	,,	30	Slight diminution. Good deflection next day.

immersed for 30 minutes in the same solution, with the result, in the first case, of diminution followed by recovery, in the second case, of abolition with no subsequent recovery. In 3617 the 30-minute bath was of the same strength as before, its effect was immediate abolition, but the nerve gave a deflection the next morning. In 3623 there was also immediate abolition, but in this case it was final. Nos. 3625 and 3627 may also be contrasted, in each case 30 minutes immersion in a 4-per cent. solution produced a slight augmentation of response, but tested the next morning the one gave a deflection and the other none. It would appear, therefore, that neurine hydrochloride is much more suitable for such experiments as these than neutralised neurine. We should mention that the frogs were in bad condition at the time these experiments were made, the weather being very hot. But this element of uncertainty would not alone account for the marked inequalities noticed.

Betaïne Hydrochloride.—We made use of two samples of this substance, one coming from Merck's factory, the other from the laboratory of Professor Boehm. The salt in each case when dissolved in 10-per cent. solution in normal saline had a strongly acid reaction, requiring for neutralisation half its volume of normal soda solution.

The effects of unneutralised solutions at 10, 5, 4, 2, and 1 per cent. are therefore partly or wholly acidic effects by 0·50, 0·25, 0·20, 0·10, 0·05 solutions of normal acid. We did not, however, work with these, but with neutralised solutions.

Method. Nerve.—The excised sciatic nerve of frog, kept not longer than 24 hours in physiological saline, is laid across two pairs of unpolarisable electrodes in a moist chamber. The normal excitatory effect (negative variation) is observed, preferably after as well as before the cut end of the nerve has been refreshed by a new transverse section. The nerve is then put to soak in the experimental solution, and from time to time replaced upon the electrodes to be tested as before. If, and when the excitatory effect is abolished, both before and after a fresh transverse section, the nerve is put to soak in physiological saline, and from time to time tested as before for a possible recovery of electromobility.

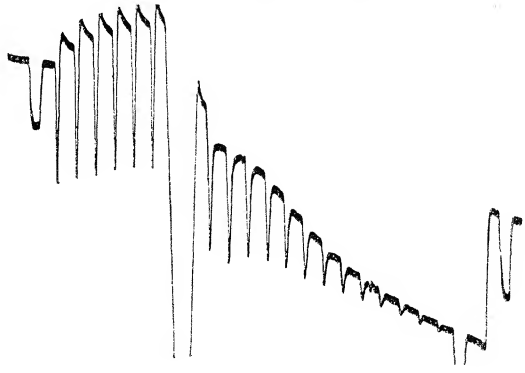
Proceeding thus we are enabled to qualify any given solution as being: 1° inert or weak, 2° moderately active, 3° strong according as the electromobility of the nerve is: 1° unaffected or little affected by the solution, 2° abolished by the solution and restored by soakage in physiological saline, 3° abolished by the solution and not restored in saline.

The circumstances of experiment, strength, and duration of excitation, distance between electrodes, are, of course, maintained unvaried. The unavoidable variations of frogs and of their nerves are far less serious than might have been expected; the nerves are removed with

ordinary care, a portion of spinal column being removed with them to serve as handles, and kept until required in physiological saline, which is best made with tap water and must not have the slightest acid reaction. The usual value of the normal response of a satisfactory nerve is between 0·001 and 0·002 volt, and the time during which a nerve may be employed for experiment is usually at the least 24—48 hours after excision. Quite fresh nerves may be used, but it is preferable to use nerves that have remained in physiological saline for an hour or two after the removal from a freshly pithed frog, and it is unadvisable to make use of nerves that have been left for any considerable length of time in the tissues of a pithed frog.

Fig. 3 (Plate 3492) gives the result of a typical experiment upon a frog's nerve submitted to the influence of a strong solution of betaïne hydrochloride (10 per cent. neutralised by half its volume of normal soda solution).

Fig. 3 (Betaïne hydrochloride).



A summary of our observations is given below; we have included in that summary for the sake of comparison seven observations on a sample of Calmette's snake venom (received from Professor Wright, of Netley), on a sample of tetanus toxine (from Mr. Plimmer, of the Lister Institute), and on some decomposed serum-albumin.

N.B.—Throughout this series of experiments the unit in which the deflection values of the current of injury and the negative variation is expressed = 0·0001 volt.

Experiment I.—Betaïne Hydrochloride. 2 per cent. solution.

First Nerve.

	In 1/10000 of a volt.	
	Current of injury.	Negative variation.
After soaking in saline for 2 hours	+25	− 4
After new transverse section	+ off scale	−15
After further 12 hours in saline	+25	−19
After new transverse section.....	+ off scale	−22
After soaking in betaïne for 20 mins.	+32	−12
After further 3 hours in betaïne.....	+33	0
After further 9 hours in saline.	+ off scale	−27
After further 10 hours in saline	+ off scale	−11
After interpolar crush.....	—	0

Second Nerve.

After soaking in saline for 1 hour	+18	− 4
After new transverse section.....	+ off scale	−25
After soaking in betaïne for 1 hour.....	+12	0
After new transverse section	+50	0
*After soaking in saline for 12 hours.....	0	−2+6
*After new transverse section	+ off scale	−33
After 1 hour in betaïne	+50	0
After new transverse section	+ off scale	0
After 12 hours in saline.....	—	−11
After new transverse section.....	—	−17

Betaïne, as regards its direct effect upon nerve, is a substance of the second class, as defined above, viz., moderately active. This conclusion is borne out by the similar results of further trials at higher and lower strengths of solution.

Exp. II.—Betaïne.

First Nerve.

		Current of injury.	Neg. var.
0 hours.	In saline for 1 hour	+25	− 5
1 „	.....	+22	− 4
	New transverse section	+ off scale	−15
12 „	In saline for 12 hours	+25	−19
	New transverse section	+ off	−22
12½ „	In bet. hyd., 2 p. c. for 25 mins....	+32	−12
16 „	In bet. hyd. for 3½ hours	+33	0
25 „	In saline for 9 hours.....	+ off	−27
35 „	Ditto for 10 hours.....	+ off	−11
	Then interpolar crush	—	0

* Plate 3503.

Second Nerve.

		Current of injury.	Neg. var.
0 hours.	In bet. hyd., 2 p. c., for 1 hour ...	+ 17	− 4
1 „	....	+ 12	0
	New transverse section	+ 70	0
12 „	In saline for 12 hours*	0	− 1, + 6
	New transverse section	+ off	− 33
12½ „	In bet. hyd., 2 p. c., for 1 hour ...	+ 54	0
16 „	New transverse section	> + 110	0
25 „	In saline for 12 hours.....	—	− 11
	Then new transverse section	—	− 17
	Then interpolar crush.....	—	0
	With reversal of excitation	—	0

Exp. III.—Betaïne Hydrochloride. 2 per cent.

Two nerves removed and placed at once in betaïne and tested 3½ hours later gave 0 response; transferred to saline and tested 9 hours later, when they gave respectively 0·0025 and 0·0011 volt. After interpolar crush these responses disappeared.

Exp. IV.—Betaïne Hydrochloride. 10 per cent.

After 16 hours in saline	− 5
After soaking in betaïne for 1 hour	0
After 4 hours in saline	− 2
Ditto after new transverse section	− 6
After interpolar crush.....	0

Exp. V.—Betaïne Hydrochloride. 1 per cent.

After 15 hours in saline	− 5
After 1 hour in betaïne	− 10
After 5 hours in betaïne	− 1
After 9 hours in betaïne	0
Ditto after new transverse section.....	0
After further 14 hours in saline.....	− 3
After further 12 hours in saline.....	− 4
Ditto after new transverse section.....	− 9
Ditto after interpolar crush	0

Exp. VI.—Muscarine Hydrochloride. 1 per cent.

	First nerve.	Second nerve.
Normal response	− 8	− 14
After soaking in muscarine solution for 5 hours; new transverse section.....	0	0
After soaking in normal saline for 8 hours	0	0

* Plate 3503.

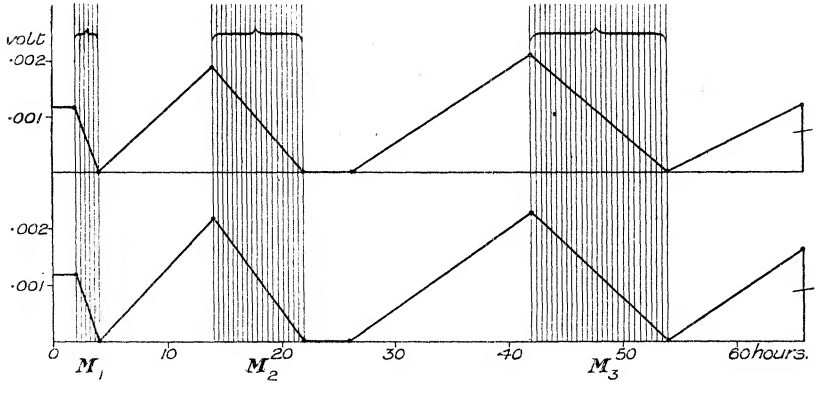
Exp. VII.—Muscarine Nitrate.

	First nerve.	Second nerve.
Normal, after $3\frac{1}{2}$ hours in saline and a new transverse section	−11	−18
After 3 hours in muscarine and new transverse section.....	0	0
After 12 hours in saline and new transverse section.....	6	6

Exp. VIII (fig. 4).—Muscarine Hydrochloride. 2 per cent. in saline.

	First nerve.		Second nerve.	
	Current of injury.	Neg. var.	Current of injury.	Neg. var.
After soaking in saline for 2 hours and a new transverse section ...	+66	−12	off +	−12
After soaking in muscarine solution for 2 hours and a new transverse section	+55	0	+66	0
After 10 hours in saline and new transverse section	off +	−22	off +	−19
After 8 hours in muscarine and new transverse section	+80	0	+60	0
After 4 hours in saline	—	0	—	0
After 16 hours in saline	off +	−23	off +	−21
After 12 hours in muscarine.....	0	0	0	0
After 12 hours in saline	—	−16	—	−12
After interpolar crush	—	0	—	0

FIG. 4.



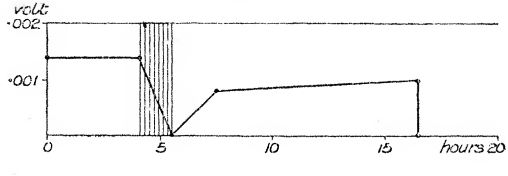
Exp. IX.—Muscarine; Betaïne; Choline; Neurine. 1 per cent.

	Current of injury.	Neg. var.
Nerve 1.—Normal	off +	− 14
After 7 hours in MUSCARINE	off +	0
After 3 hours in saline	off +	0
After new transverse section	off +	0
After further 15 hours in saline	off +	0
After new transverse section	off +	0
Nerve 2.—Normal	+ off	− 10
After 7 hours in BETAÏNE	+ 8	0
After 3 hours in saline	− 3	− 3
After new transverse section	+ off	− 20
After further 15 hours in saline	+ 30	− 10
New transverse section	+ off	− 30
Nerve 3.—Normal	+ off	− 13
After 7 hours in choline	+ 17	− 13
After further 20 hours in CHOLINE	+ off	− 4
After new transverse section	+ off	− 7
Nerve 4. — Neurine (neutralised by H_2SO_4). Normal	+ off	− 13
After 7 hours NEURINE	+ off	0
After new transverse section	+ off	− 1
After 18 hours in saline	+ off	− 2
After new transverse section	+ off	− 6

Exp. X (fig. 5).—Stale Cerebro-spinal Fluid (about 4 per cent.).

After 4 hours in saline and new transverse section	off +	− 14
After $1\frac{1}{2}$ hours in cerebro-spinal fluid	—	0
After new transverse section	—	0
After 2 hours in saline	off +	− 8
After further 9 hours in saline	+ 70	− 10
After new transverse section	off +	− 10

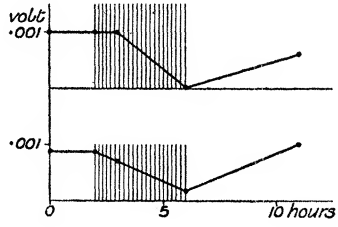
FIG. 5.



Exp. XI (fig. 6).—Snake Venom (Calmette).

	First nerve.	Second nerve.
Normal after 2 hours in saline	– 9	– 10
After 1 hour in venom	– 7	– 10
After further 4 hours in venom and a new transverse section.....	– 2	0
After 5 hours in saline and new transverse section.....	– 10	– 6

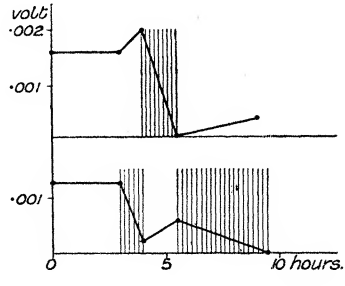
Fig. 6.



Exp. XII (fig. 7).—Snake Venom (Calmette). (Two nerves.)

Normal after 3 hours in saline and new transverse section	– 16
After 4 hours in saline and new transverse section ...	– 20
After $1\frac{1}{2}$ hours in venom and new transverse section...	0
After $3\frac{1}{2}$ hours in saline	– 4
Normal after 3 hours in saline and new transverse section	– 13
After 1 hour in venom and new transverse section.....	– 2
After $1\frac{1}{2}$ hours in saline and new transverse section.....	– 6
After 4 hours in venom and new transverse section.....	0

Fig. 7.



Exp. XIII.—Venom + Antivenom.

		(Two nerves.)
Normal after $3\frac{1}{2}$ hours in saline and new transverse section	− 14	− 14
After 11 hours in venom + antivenom and new transverse section.....	0	0
After 2 hours in saline and new transverse section.....	0	0
After further $12\frac{1}{2}$ hours in saline and new transverse section	− 10	− 10
After interpolar crush.....	0	0

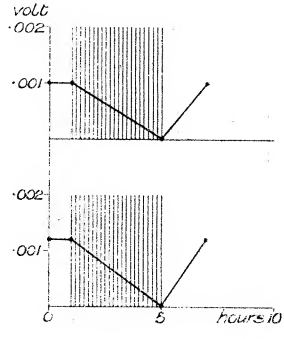
Exp. XIV.—Venom + Antivenom.

Normal after 1 hour in saline	− 10
After 1 hour in venom + antivenom and new transverse section	0
After 2 hours in saline and new transverse section ...	− 10
After further 5 hours in saline.....	− 5
Then with new transverse section	− 20
After 1 hour in venom + antivenom	− 20
After 1 hour further in venom + antivenom	− 20
After 1 hour further in venom + antivenom	0

Exp. XV (fig. 8).—Diphtheritic Toxine.

		(Two nerves.)
Normal	− 12	− 10
After bath of 4 hours and new transverse section	0	0
After subsequent bath of saline for 2 hours and new transverse section ...	− 12	− 10

Fig. 8.



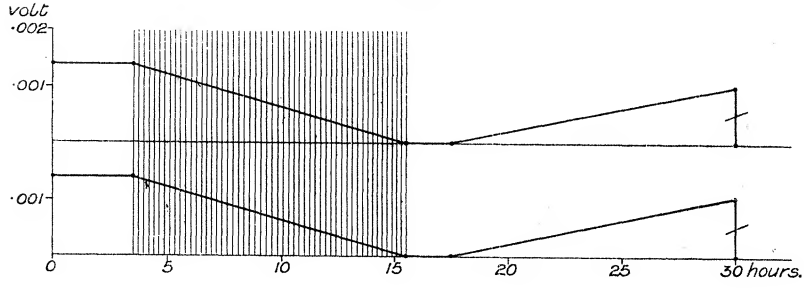
Exp. XVI.—Tetanus Toxine.

	In tetanine.			In saline.		
Normal responses of six fresh nerves.....	− 21,	− 20,	− 25,	− 16,	− 26,	− 18
After 1 hour in toxine and new transverse section.....	− 10,	− 5,	− 3,	—	—	—
Ditto control nerves in saline and new transverse section	—	—	—	− 21,	− 18,	− 21
After 3 hours in toxine and new transverse section	− 2,	0,	0,	—	—	—
Ditto control nerves in saline and new transverse section	—	—	—	− 20,	− 18,	− 20

Exp. XVII (fig. 9).—Decomposed Serum-Albumin.

	(Two nerves.)	
	First nerve.	Second nerve.
Normal after 5 hours in saline	− 11	− 10
After 1 hour in albumin and new transverse section.....	0	0
After $3\frac{1}{2}$ hours in saline	− 10	− 10
After $2\frac{1}{2}$ hours in albumin and new transverse section.....	0	0
After 13 hours in saline and new transverse section.....	− 7	− 6
After interpolar crush	0	0

Fig. 9.



Exp. XVIII (figs. 10 and 11).—Decomposed Serum-Albumin.

1. Normal series of negative variations; nerve previously kept for 3 hours in normal saline; coil at 20 units.

2. Abolition of the variation; same nerve soaked for 1 hour in a putrid solution of serum-albumin.
3. Recovery; same nerve left for 12 hours in normal saline.
4. Abolition; same nerve left for $2\frac{1}{2}$ hours in putrid serum-albumin.
5. Recovery; same nerve for $4\frac{1}{2}$ hours in putrid serum-albumin.

Fig. 10.

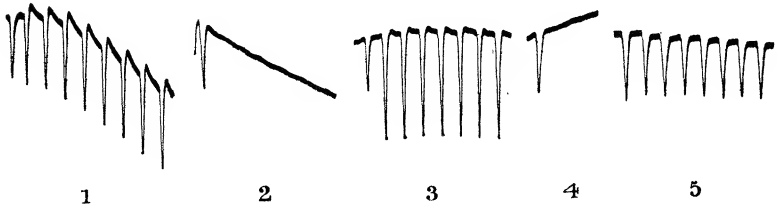
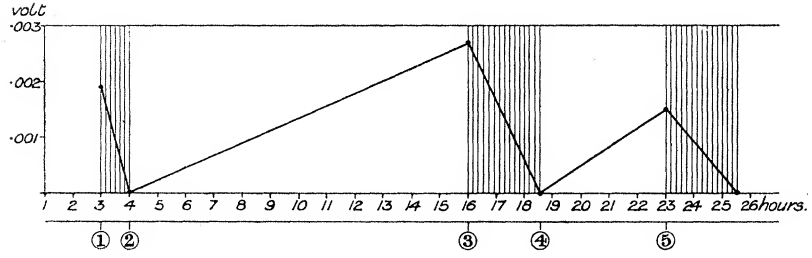


Fig. 11.



Remarks on the foregoing Experiments (I to XVIII).

Exp. I. *Betaïne Hydrochloride 2 per cent.*—This nerve exhibits abolition by betaïne and recovery by saline twice repeated.

The companion nerve exhibited a similar result twice repeated. The plotted curve (fig. 12) gives the magnitude of the negative variation.

Fig. 12.

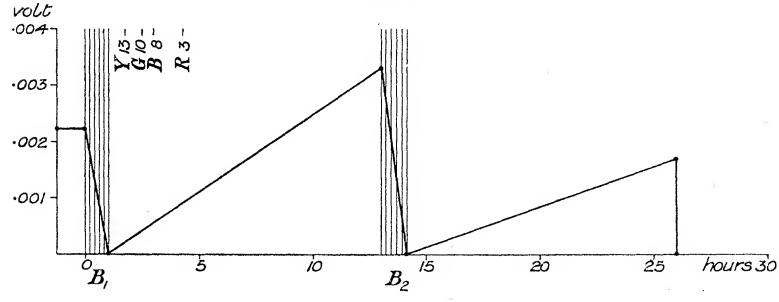


Fig. 13 is given to illustrate the refreshing effect of a new transverse section upon a small negative variation. It is not special to this particular experiment, but illustrative of a general rule of procedure that should be adopted in prolonged experiments.

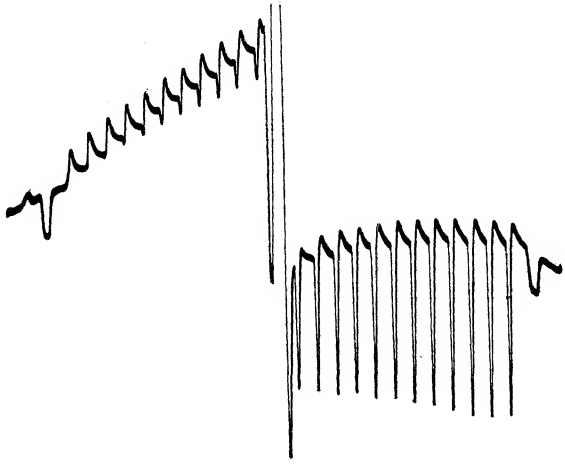
Exp. II. The two nerves of the same frog are similarly but simultaneously passed through the solutions.

From Exps. I to V we think ourselves justified in estimating betaïne as belonging to the second of the three classes specified above, *i.e.*, as "moderately active." The electromobility of nerve, as evidenced by the negative variation of its current of injury has been abolished by betaïne and restored by subsequent prolonged immersion in physiological saline.

The three following experiments show that muscarine has a similar effect, but rather more pronounced; the negative variation was permanently abolished in Exp. VI (also in Exp. IX).

Exp. VIII, in which two nerves were similarly treated, is the most complete; it exhibits in both cases abolition by muscarine and recovery by saline, three times repeated.

Fig. 13 (Exp. I).



Exp. IX. In order to make comparison as closely as possible between the individual members of the ptomaine group, we took four nerves, as nearly as possible similar, and passed them simultaneously through each of the four test solutions and through physiological saline.

The negative variation of nerve 1, immersed for 7 hours in muscarine, was completely and permanently abolished. That of nerve 2 for 7 hours in betaïne was temporarily abolished. That of nerve 3, after 7 hours in choline, was unaffected, and after 27 hours diminished.

That of nerve 4, in neurine for 7 hours, was temporarily abolished and permanently diminished.

Exp. X. The sample of cerebro-spinal fluid used in this experiment was one sent to us 3 weeks previously by Dr. Mott (and that had been tested when fresh with a negative result). On the assumption that the fluid had contained choline (which is comparatively inert), we argued that it should be found to have increased in activity in consequence of oxidation. This was found to be the case; the negative variation of the nerve was temporarily abolished by an immersion lasting 1½ hours. A similar effect is produced by the action of decomposed serum-albumin, *vide infra* Exps. XVII and XVIII.

Exps. XI to XVI were made with various toxines sent to us by Professor Wright and by Dr. Plimmer. Snake vemon (Exps. XI and XII), caused temporary abolition, and we could not, by our method of testing, find any difference of effect when the toxine was mixed with its appropriate quantity of antitoxine solution (Exps. XIII and XIV). Diphtheritic toxine (Exp. XV) and tetanus toxine also produced temporary abolition.

These few experiments, as far as they go, indicate that the toxines in question are, as regards isolated nerve tested in this manner, substances of the second degree of toxicity. But we evidently need further experiments.

The last two experiments (XVII and XVIII) were made to see whether the decomposition products of serum-albumin have a toxic action. It is evident that they have, and that their toxicity is one of the second degree, as defined above.

Experiments on the Frog's Heart.

In view of the well-known cardiac effects of muscarine, we undertook an experimental survey of the influence of the group of related bodies upon the action of the heart, taking as the most convenient case for our purpose the isolated frog's heart and the suspension method, the drug, in appropriate dilution being simply applied to the surface of the heart.

The general results of these observations were to the following effect:—

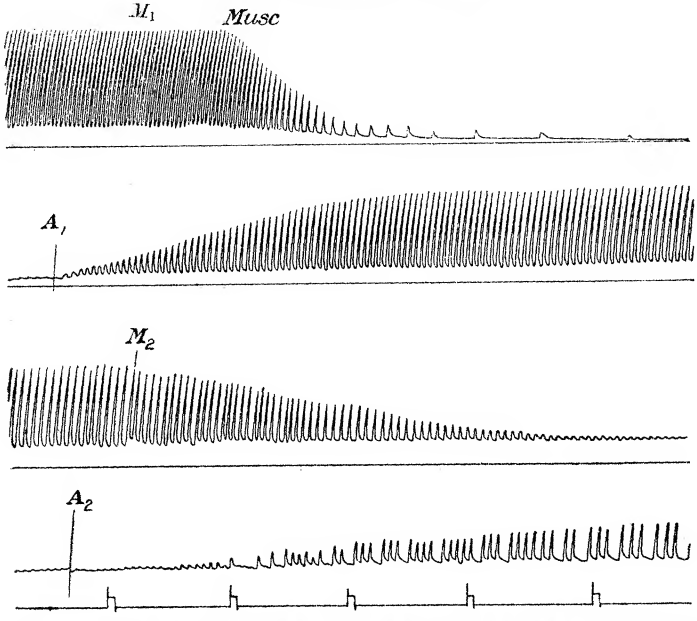
1. Neurine, muscarine, choline, betaïne (as hydrochlorides) bring about diastolic arrest of the heart.

2. The arrest thus produced is antagonised by atropine (as sulphate).

3. Neurine and muscarine are more active than betaïne or choline.

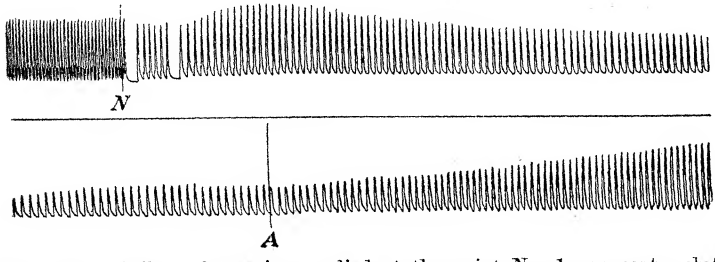
The following records are illustrative examples selected from a series of upwards of 50 experiments, all giving concordant results:—

FIG. 14.—Muscarine-Atropine.



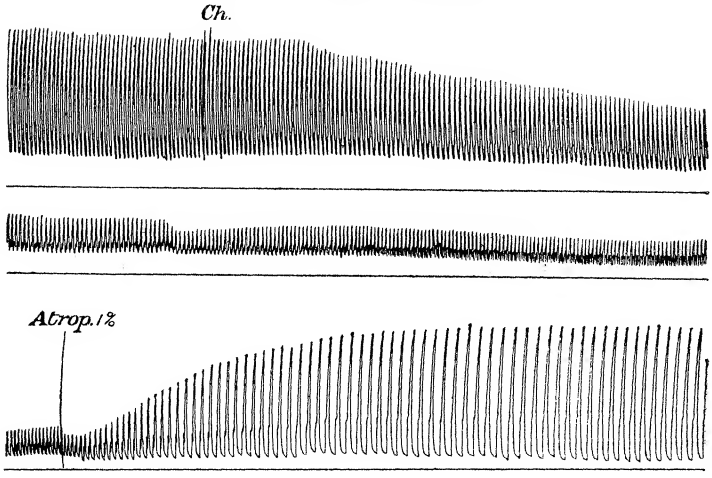
Frog's heart; suspension method. Four successive applications of muscarine hydrochloride (1 per cent. solution in normal saline) and of atropine sulphate (1 per cent.) at the points marked M_1, A_1, M_2, A_2 on the four successive lines. The record exhibits an antagonism of muscarine by atropine and *vice versâ*—*i.e.*, contrary to the usual statement, a bilateral antagonism is sometimes demonstrable between these two drugs.

FIG. 15.—Neurine-Atropine.



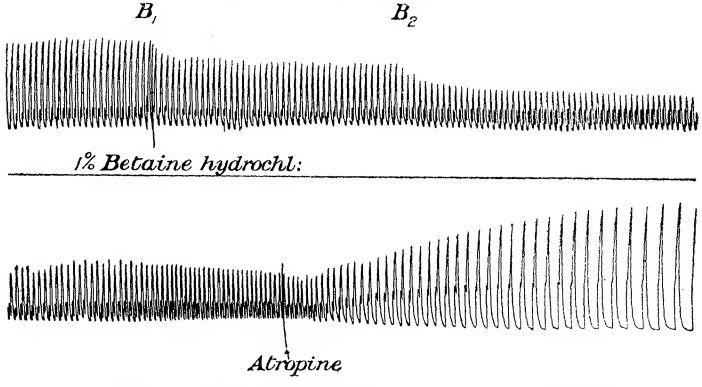
Frog's heart. Effect of neurine applied at the point N. 1 per cent. solution neutralised by H_2SO_4. Subsequent application of atropine sulphate, 1 per cent. solution, at A.

FIG. 16.—Choline-Atropine.



Frog's heart. Effect of choline hydrochloride at Ch., 2·5 per cent. solution in normal saline. Subsequent application of atropine sulphate, 1 per cent. solution.

FIG. 17.—Betaïne-Atropine.



Frog's heart. Effect of betaïne hydrochloride, 1 per cent. solution neutralised by NaOH, applied at B_1 and B_2. Subsequent application of atropine sulphate, 1 per cent. solution.

Conclusion.—The general conclusion to be drawn for the above experiments on isolated nerve and on the excised heart is that the order of toxicity of the four ptomaines examined is:—

1stly. Neurine and muscarine.
2ndly. Choline and betaïne.

The first two substances are considerably more toxic than the second two; and for each of the two pairs the first named has shown itself to be somewhat the more toxic.

As regards the excised heart, the effect of all four substances is arrest in diastole; the effect is in each case counteracted by atropine.

"The Physiological Action of Betaïne extracted from Raw Beet-Sugar." By A. D. WALLER, M.D., F.R.S., and R. H. ADERS PLIMMER, D.Sc. (Grocers' Research Student, Jenner Institute of Preventive Medicine). Received June 12,—Read June 18, 1903.

(From the Physiological Laboratory of the University of London, and the Chemical and Water Laboratory of the Jenner Institute of Preventive Medicine.)

PART 1 (A. D. W.).

From the observations described in the preceding communication, it was evident that betaïne cannot be distinguished as an inert member of the ptomaine series, at least as regards its action on isolated nerve and on the isolated heart. This led to an inquiry into the original source of the universal text-book statement that betaïne, unlike choline, neurine and muscarine, is non-toxic. The only experimental evidence to the point consists (as far as I have yet discovered*) in a statement by Schultzen, quoted by Scheibler in the 'Berichte der Deutschen Chemischen Gesellschaft' for 1870, vol. 3, p. 155, to the following effect:—

"Mehrere Versuche welche ich mit dem Betaïn ausstellte, haben übereinstimmend ergeben dass dasselbe in keiner Weise giftig wirkt, ja keinerlei wahrnehmbare Wirkungen auf das Befinden eines Thieres

* K. Andrlik, A. Velich, and Vl. Staněk, in a quite recent paper ("Das Betaïn in Physiologish-chemischer Beziehung. Vorlaüfige Mittheilung." 'Cbt. fur Physiologie,' November 22, 1902, p. 452), confirm Scheibler's statement, saying:

"Es ergab sich [an Froschen, weissen Ratten und Hunden] dass die direkte injection dieses Stoffes [Betaïn] in das Blut selbst in grösseren Mengen keine erkennbaren Aenderungen der physiologischen Functionen herbeiführt. Direkte Messungen des Blutdruckes bei curaresirten Hunden haben gezeigt, dass [das Betaïn den Blutdruck absolut nicht beeinflusst. Es war nur eine unbedeutende Pulsretardation zu vermerken. Andere sichtbare Symptome konnten nicht constatirt werden."

They injected 5 grammes *per venam* into a dog and recovered nearly the whole of this amount from the urine in an unaltered state. *Per os* only about one-third of the betaïne administered was recovered from the urine. From a cow having taken 144 grammes *per os* none was recovered.